**Stewart Postharvest Review 2015, 2:1**
www.stewartpostharvest.com
ISSN: 1745-9656

# Abscission research: what we know and what we still need to study

**Mark L Tucker* and Joonyup Kim**
Soybean Genomics and Improvement Lab, Agricultural Research Service, USDA, Beltsville, MD, USA

**Purpose of the review:** Abscission, organ separation, is an integral part of the life of a plant. Natural and artificial regulation of abscission can have substantive effects on crop yield and fruit quality. Here we present a brief overview of what we have learned about abscission in recent years as it relates to what we learned about abscission in previous decades, and also what we still need to study.
**Findings:** It has been nearly 100 years since the discovery that ethylene played a role in abscission and more than 50 years since the discovery that auxin played a role. As more hormones were discovered – cytokinins, gibberellins, abscisic acid and jasmonic acid – each has been demonstrated to have at least some effect on the timing of abscission. Auxin is of particular interest because it plays a role in cell differentiation and competence to abscise, as well as in initiation and control of cell separation. Synthesis of a protective layer at the site of organ separation is common to most abscission processes but may be regulated separate from cell separation. Study of Arabidopsis floral organ abscission has stimulated many new lines of research including work on the signaling peptide Inflorescence Deficient in Abscission (IDA) and an abscission-specific change in cytoplasmic pH that correlates with cell separation. In addition, the finding that several genes associated with the formation of organ boundaries during organogenesis in plant meristems are expressed in mature abscission zones may provide clues as to the differentiation of abscission zone cells.
**Directions for future research:** We know quite a lot about gene expression linked to cell wall disassembly in abscission but much less about earlier signals that trigger cell separation and define the cells that can respond to those signals.

**Keywords:** abscission; auxin; ethylene; adventitious; vestigial; IDA

## Abbreviations

| | |
|---|---|
| AZ | Abscission Zone |
| ***BAC1*** | *BEAN-ABSCISSION-CELLULASE 1* |
| ***CHI*** | *CHITINASE* |
| ***GUS*** | *β-GLUCURONIDASE* |
| ***HAE*** | *HAESA* |
| ***HSL2*** | *HAESA-like 2* |
| ***IDA*** | *INFLORESCENCE DEFICIENT IN ABSCISSION* |
| **JA** | Jasmonic Acid |
| ***KNOX*** | *KNOTTED-like homeobox protein* |
| **MCP** | 1-Methylcyclopropene |
| ***Nr*** | *Never-ripe* |
| ***BOP1*** | *BLADE-ON-PETIOLE 1* |
| ***BOP2*** | *BLADE-ON-PETIOLE 2* |
| ***WUS*** | *WUSCHEL* |
| ***LBD1*** | *LATERAL ORGAN BOUNDARIES DOMAIN PROTEIN 1* |
| ***BLH1*** | *BELL-like homeodomain protein 1* |
| ***Bl*** | *BLIND* |
| ***Ls*** | *LATERAL SUPPRESSOR* |
| ***GOB*** | *GOBLET* |
| ***MYB*** | *Myeloblastosis* |
| ***OVATE*** | *Ovate Family Protein* |
| ***EIN3*** | *ETHYLENE INSENSITIVE 3* |

## Introduction

The simplest definition of abscission is the act or process of cutting off. In animal biology, abscission is the last step in the separation of two daughter cells during cytokinesis [1]. In botany, abscission refers to the detachment of plant parts, eg, leaves, flowers, fruits, etc. [2**]. Separation of plant organs in the meristem during development of leaves and reproductive organs is not considered to be abscission but there are some similarities between development in the meristem and organ detachment (see below) [3]. Dehiscence, which means to split along a defined line, eg, as occurs upon the opening of a seedpod, is sometimes referred to as abscission [2**]. However, if we narrow the definition of abscission to require a fracture plane between or along a line of intact cells, which necessitates that the middle lamella be degraded, this excludes dehiscence events that occur along a line of dead cells as occurs in anthers [4].

In science we derive models based on tractable plant systems, eg, bean leaf abscission, tomato pedicel abscission, Arabidopsis floral organ abscission, etc. We then apply and test these models on a variety of different but related processes in the same plant, eg, separation of leaves, flowers or fruit, or we compare similar processes in unrelated species. A commonly cited model for abscission includes four basic steps [5-7]: first, abscission zone (AZ) differentiation; second, competence to respond to abscission signals; third, activation of abscission; and fourth, formation of a protective layer and post-abscission trans-differentiation. However, we contend that there is no unified field theory for abscission. Herein, we discuss biologi-

***Correspondence to:** Mark Tucker; Email: mark.tucker@ars.usda.gov
doi:10.2212/spr.2015.2.1

cal processes that relate to each step and reveal complexities associated with the model in describing abscission of different plant organs across the plant kingdom. Although the model has four nicely delineated steps and we will address each step separately, these basic steps are not fully independent of each other. There is considerable temporal overlap as to when each step begins and ends. Rather than examine each of the four steps sequentially, we start with step three because the actual separation event is the most obvious and extensively studied part of abscission.

### Step Three – activation of abscission – the actual separation event

Most publications and reviews on abscission focus on this stage. Because we have defined abscission as separation occurring along a layer of living cells, it is apparent that there must be expression of genes for disassembly of the middle lamella, which is the glue that binds cells together. Because the middle lamella consists of mostly pectin [8], it might seem reasonable that all that would be needed is pectinases. However, there are at least two biological reasons to express additional genes that disassemble the primary cell wall in addition to the middle lamella. First, the pore size of the primary cell wall is typically too small to allow most enzymes to readily pass through to the middle lamella that encircles the primary cell wall [8, 9]. The second reason for loosening the primary cell wall is to allow cells to expand. Cell expansion is proposed to create the mechanical forces necessary to break xylem vessels that cross the fracture plane [2**, 10, 11]. To loosen and open up the cell wall therefore requires a combination of enzymes. The primary cell wall consists of cellulose, hemicelluloses (xyloglucans, etc.), pectin and protein [8]. Although all primary cell walls contain these basic components, the chemical structure and relative concentration of the components varies. Depending on the species and organ being detached, the combination of enzymes (and non-enzymatic proteins like expansins) needed to loosen the cell wall will be different.

Although we have given two main reasons for disassembly of the primary cell wall during abscission, disassembly does not lead to a complete loss of cell wall integrity. The cells do not rupture. They remain intact. The loosening of the wall is well orchestrated involving both degradation and synthesis of new wall material. Although the cell walls of the proximal and distal separation layer cells may be similar, gene expression on either side appears to be distinct [12, 13]. This may relate to the observation that the proximal cells tend to enlarge more than the distal cells [11], which may accentuate differential forces across the separation layer that aid in separation.

It is clear that disassembly of the cell wall and middle lamella must occur, but what then regulates the expression of enzymes that disassemble this extracellular matrix? The obvious answer involves the intervention of transcription factors and regulators of transcription factors (eg, Aux/IAA, EIN3, MYB, OVATE, etc.) and possibly some post-transcriptional regulators [14]. Yes, we would expect certain families of transcription factors to be regulated similarly in many abscission processes and we are beginning to see this in studies of abscission transcriptomes [3, 15-19], but not all cell walls are compositionally the same so the regulatory network of transcription factors that are required will vary. We must then ask what regulates the changes in transcription factors? This is where we begin to look at hormones and other signals, and while there may be some commonalities among the signals used in abscission, there is no simple answer to this question that fits all the abscission processes.

It has been nearly 100 years since the discovery that ethylene played a role in abscission [20] and more than 50 years since the discovery that auxin played a role [2**]. As more hormones were discovered – cytokinins, gibberellins, abscisic acid and jasmonic acid – each has been demonstrated to have at least some effect on the timing of abscission [2**, 10, 11, 14] but changes in these may not be essential for abscission [6, 14]. More recently, the discovery in Arabidopsis of the peptide signal Inflorescence Deficient in Abscission (IDA) has stimulated a new line of research [21**-25]. Moreover, very recently, it was demonstrated that the pH of the cytoplasm of abscising cells markedly increases in multiple species [26*]. A change in pH could also be a signal.

Is there one primary signal or a set of signals common to abscission processes? Here it suffices to say that abscission can be triggered by pollination, organ maturity, ripening, disease, drought, heat, day-length, source/sink relations and more. What do most of these processes have in common? In most cases the distal organ begins a process of senescence or ripening. We typically equate senescence with an increase in ethylene [27], but also a general decline in auxin [28]. Is ethylene a common abscission signal? In most abscission systems studied ethylene clearly accelerates the abscission process [10, 14]. In the *Never-ripe* (*Nr*) ethylene-insensitive mutant of tomato the abscission of flowers is greatly inhibited [29] and treatment of wild-type tomato with 1-methylcyclopropene (MCP), an inhibitor of ethylene binding, strongly inhibits abscission [17]. In bean and soybean, we see a similar strong inhibition of abscission when ethylene antagonists are added ([30] and unpublished results). However, Arabidopsis floral organ abscission (petals, anthers and sepals) is only slightly delayed in ethylene-insensitive mutants [31, 32]. Ethylene does not appear to be essential for floral organ abscission in Arabidopsis. Moreover, petal abscission in orchids is completely independent of ethylene [33]. Collectively, these observations tell us that ethylene's role in abscission systems is not consistent.

Auxin, on the other hand, when applied distal to the AZ, strongly inhibits abscission in pedicels and petioles of tomato, bean, cotton and other species [2**, 17, 30]. Is a natural decline in auxin a universal signal for abscission? A role for auxin in floral organ abscission is not obvious [14]; however, fairly recently it was clearly demonstrated that high auxin levels delayed floral organ abscission in Arabidopsis and low auxin accelerated abscission [34]. As in other abscission processes, the antagonism of ethylene and auxin is interesting because ethylene inhibits the movement of auxin and possibly its turnover [35, 36]. Is the role of ethylene to simply inhibit the movement or metabolism of auxin? If so, in abscission processes where an increase in ethylene is not essential, as in Arabidopsis floral organ abscission, it seems plausible that the role of ethylene is the inhibition of auxin movement or acceleration of its degradation. However, in some systems, as described above for tomato and bean, ethylene responsiveness appears to be essential even after the auxin source has been removed [17, 29, 30]. In fact, a deletion analysis of the promoter for a cellulase gene induced in bean leaf abscission (*BEAN-ABSCISSION-CELLULASE 1*,

**Figure 1: Flowers from a *jointless* mutant of tomato that do not abscise but do senesce (A) and die (B) at a position within the pedicel where the joint and separation layer would normally form.**

*BAC1*) suggested that the requirement for a decline in auxin and an increase in ethylene worked on separate cis-acting elements [37].

Of particular interest in regard to abscission signals is the discovery of a secreted peptide named Inflorescence IDA, which is essential for Arabidopsis abscission [21**]. A signaling path for IDA has been proposed [22, 24]. IDA is secreted into the apoplast where it binds to the functionally redundant membrane localized receptor-like kinases HAESA (HAE) and HAESA-like-2 (HSL2), which then signals through a MAP kinase cascade to regulate changes in KNOTTED-like homeobox (KNOX) transcription factors. In Arabidopsis, IDA signaling appears to be partially independent of ethylene [31]. However, we currently do not know if auxin might influence IDA signaling in Arabidopsis abscission or, the opposite, if IDA might influence auxin signaling in abscission. Although an essential role for IDA in abscission of other species has not been demonstrated, transcripts similar to *AtIDA* do increase during the abscission of tomato leaves and flowers [38]. In fact, *IDA-like* genes have been found in every dicot genome we have examined and four monocots ([39] and unpublished results).

### Step Four – formation of a protective layer

The synthesis of a protective layer at the site of abscission serves at least two purposes. It seals the exposed surface to reduce water loss and it protects the plant from pathogen invasion. Fred Addicott's 1982 book on abscission [40] is a fantastic source of information on the anatomy and physiology of abscission. Here, we will paraphrase the vast amount of information he gives in his book. In the model we presented earlier, the protective layer is the last step. However, Addicott cites evidence indicating that synthesis of the protective layer can begin before, during or after separation commences. In herbaceous plants, which include most of the model systems we use to study abscission (tomato, bean, Arabidopsis, etc.), the protective layer forms on or around the same cells that are involved in the separation process. In perennial woody plants a more extensive protective layer forms that can include many cell layers proximal to the separation layer. Moreover, in woody plants, cell division is often observed in this proximal tissue, which can precede the separation process. In most instances, the protective layer is more extensive on the proximal side relative to the distal side. In some instances, there may be little or no synthesis of a protective layer on the distal side because there is no need to protect the abscising organ.

An increase in pathogen related gene expression was identified in AZs many years ago [41]. Recent transcriptomic studies indicate a very large component of gene expression in the AZ that might be categorized as defense related [42, 43]. Previously, it was proposed that expression of genes like *CHITINASE* (*CHI*) protected vulnerable separation layer cells from opportunistic pathogen invasion [41]. However, a part of protecting cells from pathogens is the synthesis of a protective layer around damaged cells. In an undamaged plant, the first defense against a pathogen is the cuticle [44]. We contend that in many herbaceous plants studied, the synthesis of a protective layer occurs in synchrony with degradation of the cell wall and middle lamella. Thus, synthesis of a protective layer is not really the last step but one that begins during separation and continues past the time when enzymes expressed for degradation of the middle lamella have begun to decline. Here, before moving on to another step in the model, we need to emphasize that, because the timing of cell wall disassembly and synthesis of a protective layer are not necessarily in synchrony, the signals that initiate and sustain gene expression for the two processes may not be the same.

At this point it seems prudent to visit the topic of programmed cell death (PCD). Although we defined abscission as separation along a line of living cells, PCD was authenticated in tomato abscission and citrus self-pruning [45-47]. In tomato, inhibition of PCD delayed abscission [46]. However, PCD was greatest in the distal tissue [12], which senesces. PCD is a natural component of senescence [48, 49]. It is possible that inhibition of PCD might delay abscission because the metabolism or movement of auxin in the distal tissue is altered. Moreover, in citrus, which is a woody plant, we suggest that a more extensive protective layer on the proximal side of the separation layer may isolate the separation layer cells, which might further enhance senescence and PCD of these cells.

### Step One – abscission zone (AZ) differentiation

First, we need to define what we mean by an AZ. Here, we define an AZ as a region of cells in which separation will occur. The primary AZ is a predictable separation site typically located near the base of the organ that will be abscised. In an inflorescence the AZ may be near the middle of the pedicel as occurs in tomato. The primary AZ is often morphologically distinguishable consisting of a few layers of small cells that are less vacuolated than the cells proximal and distal to the AZ [10, 11]. The separation layer within the AZ, however, may only be one or two cell layers thick and usually occurs towards the distal side of the AZ [10, 11]. There are several developmental mutants that do not make a standard primary AZ [6]. Because these are developmental mutants, they often produce pleiotropic phenotypes that affect more than just the AZ [6]. Here, we will focus on two mutants that we believe are of particular interest, the *jointless* mutant of tomato [50-53] and the *blade-on-petiole* (*bop1/bop2)* double mutant of Arabidopsis [54, 55]. The *jointless* mutant does not produce the swollen node in the middle of the flower pedicel where separation would normally occur (Fig. 1). Although the pedicels of *jointless* do not abscise, other abscission processes at the base of the leaf and at the calyx of the fruit are unaffected ([56] and unpublished results). Commercially, this is a useful trait that has been bred into many tomato varieties. When mechanically harvesting ripe tomato fruit without the *jointless* phenotype, very often the distal por-

tion of the pedicel remains attached to the fruit and can poke holes in other fruit [56]. In the *jointless* mutant the distal portion of the pedicel remains attached to the parent plant and the fruit abscises at the calyx leaving a smooth surface. Of particular interest here is that although the pedicels do not abscise and do not have the morphologically distinguishing joint, the distal half of the pedicel still senesces as would occur in a normal abscission process (Fig. 1). Why is this interesting? There are at least three possible explanations for the senescent demarcation in the *jointless* mutant. First, it is possible that the midpoint cells in the *jointless* pedicel are still somehow cryptically differentiated so that they partially respond to abscission signals arising from the distal flower or fruit. Based on the senescent phenotype of the *jointless* mutant, it appears that a protective layer might form in the middle of the pedicel independent of cell separation. Second, the entire distal half of the pedicel may be cryptically distinct from the proximal half, which allows the distal half to senesce but not the proximal half. Third, in the *jointless* mutant, an auxin gradient along the length of the petiole could evoke a senescent demarcation in the middle of the pedicel comparable to what happens in adventitious abscission (discussed below) but without cell separation between the green and senescent parts. Although Nakano *et al.* [57] compared gene expression in *jointless* and wild type pedicels, their focus was on pedicel gene expression at anthesis, which is prior to a stage at which abscission would occur. Although they did see reduced gene expression for fatty acid and lipid metabolism genes in the *jointless* background, their collection of RNA was too early to know if a protective layer would have formed between the proximal green and distal yellow (senescent) tissue.

The *JOINTLESS* gene encodes a MADS-box transcription factor [50] that is expressed in many different tissues [57]. Moreover, *JOINTLESS* gene expression is not AZ-specific but rather expressed throughout the pedicel [57]. One might predict that the JOINTLESS transcription factor regulates expression of genes that are specifically expressed in the AZ prior to abscission. Indeed, several genes that are expressed AZ-specifically prior to the induction of abscission have been identified in tomato. Surprisingly, many of these are more commonly associated with organ differentiation in apical and lateral meristems, *WUSCHEL* (*WUS*), *LATERAL ORGAN BOUNDARIES DOMAIN PROTEIN 1* (*LBD1*), *BELL-like homeodomain protein 1* (*BLH1*) *BLIND* (*Bl*), *LATERAL SUPPRESSOR* (*Ls*) and *GOBLET* (*GOB*) [3, 18]. Of interest in regard to the *jointless* phenotype is that *Bl*, *GOB*, *Ls* and *WUS* are strongly suppressed in the *jointless* background suggesting that they may play a role in the differentiation of the cells that will comprise the separation layer [57]. As mentioned above, the mutation in JOINTLESS doesn't suppress tomato leaf or calyx abscission. Thus the question that remains is whether or not these meristem-associated genes might also play a role in differentiation of the separation layer within the tomato leaf and calyx AZ where the JOINTLESS gene does not appear to play a major role.

The *bop1/bop2* double mutant of Arabidopsis is one of several developmental mutants with inhibited AZ formation [6]. *BOP1* and *BOP2*, like the genes discussed above, are highly expressed in meristems and play a role in leaf and floral organ patterning [54]. In addition, *BOP1* is strongly expressed at the base of mature Arabidopsis floral organs and leaves [55]. The *bop1/bop2* double mutant does not form the layer of small cytoplasmically dense cells at the base of the anthers, sepals and petals, and these floral organs do not abscise; nonetheless, some genes that are typically associated with floral organ abscission are still expressed, eg, *IDA* and its receptor *HAE* [55]. In addition, the promoter::reporter genes, *BAC1::GUS* and *CHI::GUS*, were also expressed in the floral AZ of the *bop1/bop2* mutant but at lower levels [55]. It would appear that BOP1 and BOP2 affect both the development of an AZ and gene expression during floral organ abscission.

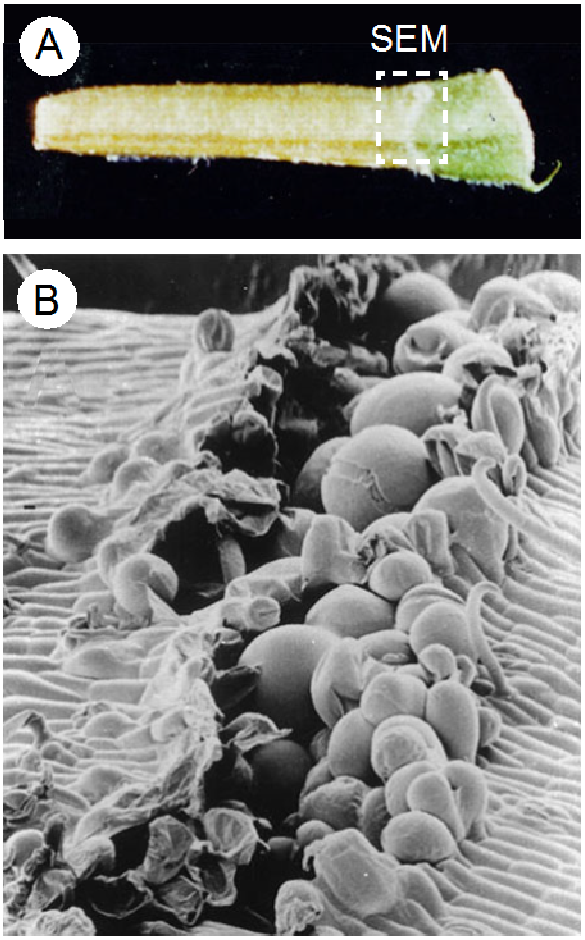
The phenotype of *bop1/bop2* mutant is of further interest here because it also relates to a particular abscission topic that we want to briefly introduce, vestigial AZ [55]. A vestigial AZ is a latent AZ at the base of an organ where abscission might be expected to occur but usually does not. For example, the rosette and cauline leaves of wild-type Arabidopsis do not normally abscise. In Arabidopsis, *HAE*, one of the redundant receptors for IDA, is expressed at the base of rosette [58] and cauline [59] leaves and flower pedicels [59] and yet, as defined by vestigial, these organs do not typically abscise. However, when IDA was constitutively over-expressed in Arabidopsis using a 35S promoter, a small cleft formed at the base of branches, cauline leaves and pedicels [60*]. The cleft consisted of slightly swollen irregular shaped cells. This phenotype suggested to the authors that a partial abscission process had begun in the vestigial AZ [60*]. Interestingly, when the IDA over-expression genotype was introduced into the *bop1/bop2* double mutant, the cleft in the axes of cauline leaves was not observed, suggesting that the partial abscission phenotype of the IDA over-expression line did not occur [55]. Although, as mentioned above, the *HAE* gene was expressed in the floral AZ of the *bop1/bop2* double mutant, the authors did not mention whether or not the *HAE* gene was expressed in the vestigial AZ of the *bop1/bop2* mutant [55]. Although there may exist many possible explanations for why partial abscission did not occur in this mutant, the lack of a receptor for IDA could be one explanation. Collectively, these observations accentuate that multiple signals and differentiation events must occur to produce a fully competent AZ, and we are just beginning to understand how meristem-associated genes might play a role in defining both the AZ and the separation layer within the AZ.

## Step Two – competence to respond to abscission signals

What makes an AZ competent to respond to abscission signals? The simple answer is a decline in auxin, which will be reviewed in greater detail in a separate article [Meir *et al.,*] in this issue. Nonetheless, auxin needs to be discussed briefly here. It is often said that a decline in auxin sensitizes the AZ to ethylene [14, 17]. Generally, if the concentration of auxin is maintained at a high level either because of an actively growing distal organ or exogenous application of auxin, ethylene will not induce abscission [11, 17, 30]. However, if the distal organ is removed and is not replaced by an exogenous source of auxin, abscission is induced or accelerated by ethylene.

Although we have consistently referred to the decline in auxin as being necessary to allow abscission to occur, there are other interpretations. Many years ago, Louie and Addicott [61*] published very interesting results related to cotton petiole abscission. Using a stem-petiole explant, they obtained the expected

**Figure 2. Adventitious abscission in a bean (*Phaseolus vulgaris*) petiole induced by treating the petiole stump with 1 mM IAA (A), and (B) scanning electron micrograph (SEM) of the separation layer outlined in A above. Reprinted from [64].**




inhibition of abscission when they applied auxin to the distal end of the cut petiole; however, when auxin was applied to the proximal stump of the stem, this accelerated abscission. They proposed that it was the reversal of the gradient across the AZ that was important for the induction of abscission. More interestingly, when they applied auxin to both the distal and proximal stumps at different concentrations and ratios, they found that it was not the concentration that mattered but the ratio that determined the rate of abscission in the primary AZ. They proposed that the magnitude of the auxin gradient across the AZ was the important factor and not the absolute amount of auxin.

Another important question in regard to establishing abscission competence is whether or not the entire separation layer is predifferentiated to respond to an external abscission signal, or, alternatively, are just a few cells within the separation layer differentiated to respond to the external signal (target cells) and then these cells radially secrete a secondary signal outwards? Often the separation layer cells are not distinguishable from the surrounding AZ cells [2**]. It makes sense that there might be only a few cells that perceive, for example, the decline in auxin or reversal of the auxin gradient and these target cells signal others to separate. In fact, Thompson and Osborne [62**] demonstrated that in bean leaf abscission a diffusible signal emanated from the vascular tissue to induce separation in the cortex cells. However, Sexton [63**] arrived at a different conclusion when studying leaf abscission in Impatiens. He demonstrated that a cross-section of the petiole AZ could be dissected into many small pieces prior to receipt of any abscission signals and each piece would subsequently undergo cell separation independent of the others. He concluded that cell-to-cell contact within the AZ was not required and the separation layer cells across the entire petiole were pre-differentiated to abscise. Much later, we revisited this question for tomato leaf abscission using transgenic plants expressing GUS driven by an abscission -specific polygalacturonase gene promoter [38]. We found that, when the cortical cells of the AZ were dissected away from the vascular cells and exposed to ethylene, they separated into proximal and distal parts and expressed GUS in the separation layer cells independent of the vascular tissue. For tomato leaf abscission, it was concluded that the cortical cells are predifferentiated to abscise [38].

To make it even more interesting, we will add a discussion of differentiation and signaling associated with adventitious abscission. Adventitious abscission occurs in unpredictable and presumably undifferentiated places in the plant, eg, the middle of the stem or petiole [64**-72**]. If a stem or petiole explant is simply left to age in a moist environment, internodal adventitious abscission can occur [64**, 72**]. However, if auxin is applied to the base of the stem or petiole, the onset of adventitious abscission is greatly accelerated [64**, 72**]. Somewhat different from the observations by Louie and Addicot of the primary AZ of cotton [61*], the concentration of auxin applied to the stems of Impatiens affected where the adventitious separation occurred along the length of the stem [64**, 69, 71, 72**]. In other words, higher concentrations of auxin applied to the base moved the adventitious abscission further up the stem. McManus *et al.* [64**] confirmed these results with petiole explants of bean, *Phaseolus vulgaris*. Similar to the study of Impatiens described above, the position along the bean petiole where the adventitious separation occurred was dependent upon the concentration of auxin applied; moreover, cell division was not required for induction of adventitious abscission. Ultrastructural examination of the adventitious separation layer indicated that it included enlarged swollen cells very similar to those seen in the separation layer of the primary AZ ([64**] and Fig. 2).

It would appear that cells in the primary AZ somehow sense only the reversal of the auxin gradient, but for transdifferentiation of adventitious AZ both an auxin gradient and auxin concentration is sensed. The separation layer in both a primary AZ and an adventitious AZ is comprised of many cell types, eg, phloem, vascular parenchyma cells, cortical cells, etc. It seems simpler if only a few cells respond to the change in auxin gradient and then produce a separate abscission signal that radiates outwards, but work in Impatiens [63**] and tomato [38] indicate that in a primary AZ the separation layer cells are predetermined to respond to abscission signals. A question that comes to mind is, does an auxin gradient form across the dissected pieces of AZ tissue, as used in the study of Impatiens and tomato abscission, or is it simply a low concentration of auxin and the synthesis of ethylene that is required to initiate separa-

tion in the AZ dissections? This is a difficult question to address. Determining the absolute concentration of auxin in a single cell or a few cells is not a simple task.

## Conclusions

No single abscission model fits all forms of abscission. Cell wall disassembly is the one feature common to all separation events, but how these cells differentiate to be competent to respond to abscission signals is more varied. Auxin seems to be important in most primary and adventitious abscission systems but understanding how cells might sense an auxin gradient is still elusive. Floral organ abscission in Arabidopsis has been a useful system to identify and characterize IDA and HAESA signaling and other determinants of abscission regulation, but the role of these regulators in other species needs to be examined further. The finding that the cytoplasmic pH of AZ cells increases during abscission in three species, two of which were closely related, is interesting and needs to be examined further. The timing of the synthesis of a protective layer in primary abscission and the cells involved are variable among species and the type of organ being abscised. Because the synthesis of a protective layer would affect water relations and the extracellular matrix of separating cells, the synthesis of a protective layer would be expected to modulate the rate of organ separation. What are the signals for synthesis of a protective layer? Are they the same or different from cell separation signals? Is a protective layer synthesized during adventitious abscission? Also, are the meristem-associated genes discovered in tomato pedicel AZ expressed in adventitious AZs as well? The meristem-associated genes may be required for the transdifferentiation of separation layer cells in the adventitious abscission described by McManus *et al.* [64**]. We know a lot about abscission, but we still have much to learn.

## Acknowledgements

Support for JK comes from a BARD grant (US-4571-12C) to MLT. We would like to thank Brad M. Binder for critical reading of this manuscript.

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